

No actual conflict over colony inheritance despite high potential conflict in the social wasp *Polistes dominulus*

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Social insect societies are outstanding examples of cooperation and conflict. Individuals work together, yet seek to increase their inclusive fitness at each others' expense. One such conflict is over colony inheritance, when a queen inherits the colony following the death of the previous queen. Colony inheritance is common in the social wasp *Polistes dominulus*, and it can have dramatic fitness consequences. The subordinate inheriting the colony is often unrelated to the initial foundress (alpha) and the workers, who therefore get zero inclusive fitness. Workers are capable of mating and reproducing, so that inheritance by a subordinate rather than by a related worker is surprising. Using patterns of egg-laying and egg destruction, we show in 32 laboratory colonies that, upon the removal of alpha, workers fully accepted a subordinate as the new breeder. This new alpha monopolized reproduction to the same extent as alpha, and there was no increase in reproduction by workers and other subordinates. Why workers accept a potentially unrelated subordinate as breeder rather than a full-sister worker is unclear. They may be constrained to do so, and they may seek fitness benefits by producing males later in the season or by absconding the nest.

Keywords: supersedure; queen replacement; inclusive fitness; egg laying; egg destruction

1. INTRODUCTION

A primary characteristic of animal societies is cooperation among group members, and insect colonies stand out as societies where cooperation is highly advanced. Nonetheless, conflicts of interests are common in social insects (e.g. Heinze *et al.* 1994; Monnin & Ratnieks 2001; Ratnieks *et al.* 2006; Strassmann & Queller 2007), and one such conflict is over colony inheritance. In some species, the dying queen is replaced by another queen that effectively inherits the colony, i.e. the nest with the brood and the workforce. The replacement queen who inherits the colony greatly benefits from this (e.g. Sanetra & Crozier 2002), but conflict may arise with other putative replacement queens who also seek to inherit the colony, and with workers who may favour another queen inheriting. Conflict may be overt, with potential replacement queens fighting and/or workers favouring and helping one queen over another (e.g. *Apis mellifera*; Dietemann *et al.* 2008; Schneider & DeGrandi-Hoffman 2008). Conflict may also be covert, with the replacement queen failing to gain the full cooperation of workers, who may produce males or abscond the nest (e.g. Tibbetts 2007).

Colony inheritance has major fitness consequences and occurs in many social insects. For instance, it occurs in all species reproducing by colony fission (e.g. honeybees, army ants, queenless ants). It is also common in species where queen mortality is high during colony foundation,

such as polistine wasps. We investigate potential conflicts over colony inheritance in the primitively eusocial wasp *Polistes dominulus*, where colony inheritance is an important life-history trait. In *Polistes* species from temperate regions, such as *P. dominulus*, several foundresses often associate to initiate the colony in early spring. Reproduction is unevenly shared, with a dominant foundress (alpha) more or less monopolizing reproduction, while subordinate foundresses behave as workers (e.g. Reeve 1991; Johnstone 2000; Queller *et al.* 2000; Reeve & Keller 2001; Nonacs *et al.* 2004; Liebert & Starks 2006; Nonacs 2007). The first brood gives rise to workers, who are daughters of alpha and are capable of mating and reproducing in orphaned colonies. Because foundress mortality is high, a subordinate commonly inherits the colony following the death of alpha (Strassmann 1981; Strassmann & Meyer 1983; Hughes *et al.* 1987; Strassmann 1989; Reeve 1991; Peters *et al.* 1995; Queller *et al.* 1997, 2000; Reeve *et al.* 2000; Cant & Field 2001; Strassmann *et al.* 2003).

Colony inheritance has important fitness consequences for alpha, subordinate(s) and workers, and conflicts of interests may arise. Subordinates may be sisters of alpha, but they may also be unrelated (Queller *et al.* 2000; Reeve *et al.* 2000; Cant *et al.* 2006; Zanette & Field 2008). Queller *et al.* (1997) showed that subordinates would benefit from becoming reproductive, either by overthrowing alpha or by inheriting the colony, whereas alpha opposes being superseded. Workers should also be opposed to a subordinate inheriting the colony. They would benefit most by retaining alpha as breeder, and if alpha dies they should favour a sister worker becoming reproductive rather

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than a subordinate. This is especially so, given that subordinates may be unrelated to alpha and hence to workers, who would gain zero fitness by working for an unrelated subordinate. How can a subordinate inherit the colony despite workers and alpha having a common interest in preventing colony inheritance by a subordinate?

Alpha and/or workers may force subordinates to leave the colony in order to prevent them from inheriting the colony. Evidence for this is that subordinates tend to disappear from the nest around the time of worker emergence (Pfennig & Klahn 1985; Hughes & Strassmann 1988; Reeve 1991; Reeve *et al.* 1998; Gamboa *et al.* 1999), even though they are unlikely to leave of their own volition. However, a subordinate apparently inherits the colony if alpha dies before subordinates have left the colony. This raises the question of whether this subordinate effectively inherits the colony: although she becomes behaviourally dominant, she may not monopolize reproduction to the same extent as alpha. Workers may detect that supersedure has occurred, and they may work less and seek direct fitness benefits by producing males or by absconding the nest (e.g. Tibbetts 2007). If a subordinate effectively inherits the colony, why workers accept a subordinate as breeder remains an unanswered question.

We experimentally tested whether *P. dominulus* subordinates and workers are in conflict over colony inheritance and how this conflict may be resolved. We investigated whether a subordinate effectively inherits the nest upon the removal of alpha in colonies where workers are already present. Specifically, we determined whether the subordinate who becomes dominant upon the removal of alpha (the new alpha) lays as many eggs as alpha and inhibits subordinate and worker reproduction as successfully as alpha. In order to do this, we compared egg-laying and egg destruction in colonies with a new alpha with those in colonies where alpha monopolized reproduction on the one hand, and with those in colonies where alpha did not monopolize reproduction on the other hand. We also investigated whether relatedness between foundresses affects colony inheritance. Finally, we analysed the effect of colony size on egg-laying. Workers may be constrained not to reproduce in small colonies, where the cost of worker reproduction may be prohibitive in terms of decreased work and colony efficiency.

2. MATERIAL AND METHODS

(a) Sampling of colonies

Thirty-two colonies of *P. dominulus* with several foundresses were collected from a site near Paris in early June 2005 (2 colonies), and a second site near Florence in early June 2005 (10 colonies) and early June 2006 (20 colonies). Colonies were collected before worker emergence (i.e. with no uncapped cells) to ensure that no large worker would be mistaken for a foundress and no small foundress would be confused with a worker. Foundresses were individually marked with large colour dots on the thorax immediately after nest collection. Colonies were maintained in the laboratory and the experiment started 9–15 days after nest collection, when colonies had 19 ± 10 females (foundresses + workers).

(b) Experimental treatments

Dominance ranks between foundresses were identified by monitoring aggressive interactions, presence on the nest comb and egg-laying during 2 days preceding the experiment. The identification of alpha was absolutely unambiguous. She laid most eggs, she remained most of the time on the comb and she frequently lunged towards other foundresses (e.g. Strassmann *et al.* 2004). The ranking of subordinate foundresses was less reliable, though beta was relatively frequently present on the comb of the nest while other subordinates were seldom observed on the comb. In our experiment, we pooled the subordinates from each colony (see below), so that ranking subordinates precisely was not required.

Colonies were assigned to three groups. In 'alpha-removed' colonies ($n=8$ from 2006), the alpha foundress was removed from the nest at the beginning of the first day of the experiment. As a consequence of this removal, subordinate foundresses gained one rank in the dominance hierarchy such that one subordinate became the new alpha. This treatment allows quantification of the reproduction achieved by this new alpha, by measuring the egg-laying rates of the new alpha, subordinates and workers, and the destruction rate of new alpha-, subordinate- and worker-laid eggs. 'Control' colonies were not manipulated ($n=5$ and 6 from 2005 and 2006, respectively). They allow quantification of the alpha's reproduction in unmanipulated colonies where alpha monopolizes (or nearly monopolizes) reproduction. In 'brood-removed' colonies ($n=7$ and 6 colonies from 2005 and 2006, respectively), worker reproduction was experimentally triggered by regularly removing brood from the nest (Liebig *et al.* 2005, see details below). This permits the quantification of alpha's reproduction when she fails to monopolize reproduction. Therefore, the comparison of alpha-removed colonies with control and brood-removed colonies (i.e. colonies where alpha does and does not monopolize reproduction, respectively) allows the investigation of whether the new alpha monopolized reproduction or not: that is, whether she becomes as fertile as alpha or not, and suppresses subordinates and workers from reproducing as efficiently as alpha does or not. This comparison allows determination of whether the new alpha effectively inherits the colony or not.

Colonies from the three groups were cooled down in a fridge for approximately half an hour every other day for 22 days, on the morning of every odd day. All wasps were removed from the nest, and the comb was mapped to survey the addition of new cells and the fate of cell contents. In brood-removed colonies, the brood was removed from 50 per cent of the cells, excluding those forming the outer ring of the comb, following Liebig *et al.* (2005). In the three treatments, wasps were then returned to the nest and filmed to monitor egg-laying.

Only colonies with three or more foundresses were assigned to the alpha-removed treatment, so that at least two foundresses would remain after the removal of alpha. Because of this, alpha-removed colonies had more foundresses at the time of collection than the colonies in the other two treatments, but this difference was not significant: alpha-removed colonies had 4.0 foundresses (median), while both control and brood-removed colonies had 3.0 foundresses (quartiles = 3.0 and 5.0, 2.5 and 4.0, and 2.0 and 3.0, respectively, Kruskal–Wallis ANOVA: $H_{2,32}=5.595$, $p=0.060$). As a result of the removal of alpha, alpha-removed

colonies also had 3.0 foundresses at the beginning of the experiment (quartiles = 2.0 and 4.0, Kruskal–Wallis ANOVA: $H_{2,32} = 0.894$, $p = 0.639$).

Beta accounted for 78.6 per cent of subordinate foundresses reproduction, and for simplicity we pooled all subordinates' reproduction. Colonies from the three groups had equal worker and cell numbers at the beginning of the experiment: control, brood-removed and alpha-removed colonies had 17.0, 13.0 and 22.5 workers (median, quartiles = 13.0 and 29.0, 8.0 and 27.0, and 17.2 and 26.2, respectively, Kruskal–Wallis ANOVA: $H_{2,32} = 1.689$, $p = 0.429$), and their nests had 94.0, 80.0 and 81.0 cells (quartiles = 73.5 and 99.5, 52.0 and 97.0, and 71.2 and 100.7, respectively, Kruskal–Wallis ANOVA: $H_{2,32} = 1.035$, $p = 0.596$).

(c) Egg-laying

Egg-laying is a conspicuous behaviour in *Polistes*, and is easily identifiable on video. The laying wasp inserts her abdomen deep into a cell, with her hindlegs spreadeagled and her wings partially stretched out, and she remains motionless for a minute or more. Egg-laying was quantified by continuously video recording the comb of the nest for 2 days every 4 days, i.e. during days 1+2, 5+6, 9+10, 13+14, 17+18 and 21+22 of the experiment (see Liebig et al. 2005). These periods of video recording are hereafter referred to as sessions 1–6, respectively. Recording was carried out at approximately 4 frames per second, using Sony Digital 8 camcorders connected to Panasonic AGTL750 time-lapse video recorders. Weak indirect lighting was used at night, to allow the recording of egg-laying in semi-darkness. Occasionally, video recordings failed because the wasps had dirtied the glass window through which the comb was filmed or the night light failed overnight. Nevertheless, the sampling effort remained equivalent for the three groups: 278 hours for both control and brood-removed colonies, and 283 hours for alpha-removed colonies (median, Kruskal–Wallis ANOVA: $H_{2,32} = 4.445$, $p = 0.108$).

(d) Egg replacement

Although several eggs are routinely deposited in the same cell over the course of a few hours, it is uncommon to find two eggs in a cell. This shows that egg destruction is intense. Egg replacement may drastically affect the reproductive apportioning between alpha, subordinates and workers, so we quantify this by calculating an index of replacement for each party of interest. For every day (i.e. each videotape), we recorded the cells where one or more eggs were observed being laid, and we identified for each of these cells whom from (new) alpha, subordinates and workers deposited the first and last eggs. The index of replacement is the number of last-laid eggs minus the number of first-laid eggs. The former are the shares of reproduction secured after replacement, while the later are the shares before replacement. The index of replacement is positive when one benefits from replacement, negative when one suffers from replacement and nil when no replacement occurs or when the replacement egg is laid by the same individual. For each day, the replacement indices of (new) alpha, subordinates and workers sum to 1, because any increase in reproductive apportioning is at the expense of another party of interest.

(e) Genetic analysis

We estimated relatedness between co-nesting foundresses in the 30 nests collected near Florence, using seven highly

polymorphic microsatellite markers specifically developed for *P. dominulus* (Pdom 7, Pdom 20, Pdom 117, Pdom 121, Pdom 122, Pdom 127b and Pdom 140; Henshaw 2000). Dead or dying foundresses were collected and preserved in alcohol throughout the experiment, and entire colonies were frozen at -80°C at the end of the experiment. The genetic analysis failed for some of the foundresses found dead, because of decayed DNA, so that the sample slightly differs between behavioural and genetic analyses. DNA was extracted from the abdomen with Qiagen DNA tissue kit QIAquick 96, following the manufacturer's protocol, and diluted in 150 μl elution buffer. Each PCR was run in a 10 μl volume containing 1 μl DNA solution (10–40 ng DNA), 150 μM of each dNTP, from 80 to 140 nM of each primer (80 nM for Pdom 7 and Pdom 20, 100 nM for Pdom 140, 120 nM for Pdom 121, Pdom 122 and Pdom 127b; 140 nM for Pdom 117), $1\times$ Taq buffer and 0.75 units of Taq DNA polymerase (Q Biogen). Thermocycle conditions were as follows: 10 min at 94°C followed by 10 amplification cycles at 94°C for 15 s, 54°C for 15 s and 72°C for 30 s; 20 amplification cycles at 89°C for 15 s, 54°C for 15 s and 72°C for 30 s; and a final elongation step of 10 min at 72°C . Amplification products were loaded on an ABI PRISM 310 sequencer (Applied Biosystems) and allele sizes were estimated using the GENESCAN software. Two sets of loci were co-amplified (first multiplex: Pdom 7, Pdom 20, Pdom 140; second multiplex: Pdom 121, Pdom 122, Pdom 117, Pdom 127b).

Genetic relatedness among co-nesting foundresses was estimated for each colony and averaged over colonies, using RELATEDNESS v. 5.1 software, weighting the colonies equally (Queller & Goodnight 1989). Standard errors of the means were obtained by jackknifing over colonies. Deviation from the Hardy–Weinberg equilibrium was tested using GENEPOP WEB v. 3.4 for a dataset including only one randomly selected individual per colony. One locus (Pdom 20) showed a significant excess of heterozygotes ($F_{is} = 0.109$, $p = 0.007$), but this deviation was not significant after Bonferroni correction. With all loci combined, there was a marginally significant deviation from the Hardy–Weinberg equilibrium ($\chi^2_{14} = 23.5$, $p = 0.05$), mainly due to the locus Pdom 20. There was therefore no sign of inbreeding in our sample.

From the allelic frequencies estimated by RELATEDNESS, the mean level of gene diversity (or expected heterozygosity) over the seven loci was 0.86 (range: 0.72–0.93). The aim of our genetic study was to test whether pairs of co-nesting foundresses were related or not. We estimated pairwise relatedness among co-nesting foundresses using RELATEDNESS v. 5.1. We also estimated the expected distribution of pairwise relatedness, given our allelic frequencies using KINSHIP v. 1.2, assuming that individuals were either unrelated ($r_p = r_m = 0$) or full sisters ($r_p = 1$, $r_m = 0.5$). We ran 1000 simulations, using the allelic frequencies obtained from RELATEDNESS and weighting colonies equally. We also used COLONY v. 1.2 to assign individuals to full-sib families (Wang 2004). This software estimates population allele frequencies simultaneously with the reconstructed sibships and can account for typing errors (we assumed a rate of 0.05 for each locus).

(f) Statistical analyses

The number of eggs laid per session was transformed as log (number of eggs + 1) and analysed with a repeated-measures ANOVA, using STATISTICA v. 7.1. Treatments (control, brood removed, alpha removed) and social status ((new) alpha, subordinates, workers) were used as factors, and the number

of eggs laid per session was used as the repeated measure (six repeated measures). The indices of egg replacement could not be normalized and were therefore analysed with non-parametric statistics. For each treatment, social status was compared within colonies by Friedman ANOVA and Wilcoxon signed-rank tests. Between treatments, social status was compared between colonies by Kruskal–Wallis ANOVA and *post hoc* tests. The potential correlation of colony size with egg-laying was analysed by means of Pearson's r .

3. RESULTS

(a) Genetic analysis

The genetic relatedness among co-nesting foundresses of the 30 colonies collected in the population near Florence was 0.23 ± 0.057 (mean \pm s.e.). This is significantly lower than the expected relatedness of 0.75 among full sisters (t -test = 9.15, $p < 0.0001$), and significantly higher than zero (t -test = 4.0, $p = 0.0001$). Relatedness was clearly bimodal, with one peak near 0 and the other near 0.75 (figure 1). These two peaks fit remarkably well with the expected distributions of pairs of unrelated individuals and pairs of full sisters, given the genetic diversity of our microsatellite markers. This suggests that our co-nesting foundresses are a mixture of unrelated individuals and lowly related relatives, such as cousins (first peak), and of full sisters (second peak). Therefore, we assigned pairs with relatedness lower than 0.4 to unrelated foundresses and pairs with relatedness higher than 0.6 to full-sister foundresses. Three pairs with relatedness between 0.4 and 0.6 could not be assigned this way. A likelihood analysis, using COLONY v. 1.2, confirmed all our previous assignments and assigned the above three pairs to full sisters. Overall, 15 colonies contained only unrelated foundresses (six control, seven brood-removed and two alpha-removed colonies), five colonies contained only full-sister foundresses (two control, one brood-removed and two alpha-removed colonies) and six colonies contained a mixture of both (one control, one brood-removed and four alpha-removed colonies). Given that the number of colonies with related versus unrelated foundresses was so unbalanced, the potential effect of relatedness on egg-laying could not be tested.

(b) Experimental treatments

Colonies from the three groups produced equal numbers of adults over the 22 days of the experiment. Control, brood-removed and alpha-removed colonies produced 10.0, 11.0 and 11.5 females (median, quartiles = 7.0 and 18.0, 8.0 and 13.0, and 10.0 and 13.2, respectively, Kruskal–Wallis ANOVA: $H_{2,32} = 0.616$, $p = 0.734$), and 3.0, 1.0 and 1.0 males (quartiles = 1.5 and 9.5, 1.0 and 3.0, and 0.0 and 6.0, respectively, Kruskal–Wallis ANOVA: $H_{2,32} = 2.457$, $p = 0.292$). Colonies from the three groups differed in the number of cells they added to their nests (Kruskal–Wallis ANOVA: $H_{2,32} = 10.392$, $p = 0.005$). Brood-removed colonies added 0.0 cells (median, quartiles = 0.0 and 1.0), which is significantly fewer cells than control colonies (19.0, quartiles = 13.5 and 29.5, *post hoc* test: $p = 0.006$), but not significantly fewer than alpha-removed colonies (8.5, quartiles = 4.5 and 14.5, $p = 0.124$). Control and alpha-removed colonies did not differ in the number of cells they built over the duration of the experiment ($p = 1$). This pattern was

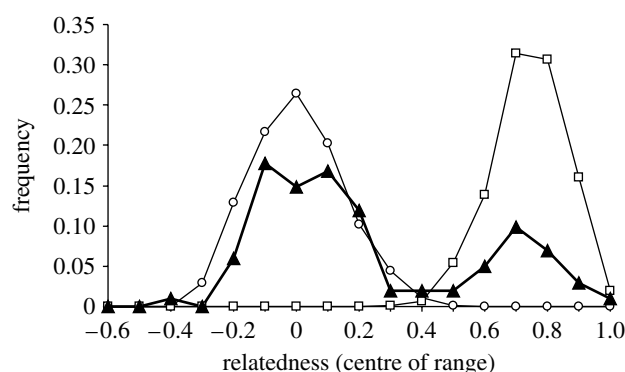


Figure 1. Relatedness distribution of pairs of *P. dominulus* foundresses. Triangles, observed distribution for 101 pairs of co-nesting foundresses; circles, expected distribution for simulated pairs of unrelated individuals ($r=0$); squares, expected distribution for simulated pairs of full-sister individuals ($r=0.75$). The expected distributions are based on 1000 runs performed with KINSHIP v. 1.2 using the allelic frequency of our microsatellite loci. See §2 for details.

expected: *P. dominulus* build new cells in order to lay eggs (e.g. Strassmann *et al.* 2004), and building new cells was unnecessary in brood-removed colonies where many cells were regularly emptied and thus available for egg-laying.

(c) Egg-laying

We video recorded the deposition of 5140 eggs in 3072 cells. The number of eggs laid differed between treatments, with more eggs being laid in brood-removed colonies than in either alpha-removed or control colonies (table 1). The number of eggs laid also varied with social status, with (new) alpha laying more eggs than either the subordinates or the worker collective (table 1). The treatment \times status interaction was not significant (table 1), indicating that the pattern of egg-laying was similar in the three treatments: (new) alpha was the main egg layer, followed by the worker collective and by subordinates (figure 2). The number of eggs laid increased over time, as already shown by Liebig *et al.* (2005), with more eggs deposited from the second session onwards (table 1). There was a significant treatment \times time interaction (table 1). Egg-laying increased dramatically in brood-removed colonies from session 2 onwards, while it increased slightly in sessions 5 and 6 in alpha-removed colonies, and it did not increase in control colonies. Similarly, there was a significant status \times time interaction (table 1), because egg-laying increased over time for workers while it was relatively constant for the (new) alpha and subordinates.

Overall, these results show that alpha-removed colonies did not differ from control colonies, but did differ from brood-removed colonies. Following the removal of alpha, the new alpha laid as many eggs as alpha, and inhibited worker and subordinate reproduction to the same extent as alpha.

(d) Egg replacement

Of the 3072 cells in which eggs were observed to be deposited into, 64.5 per cent received one egg and 35.5 per cent received several eggs (20.2% received 2 eggs, 7.6% received 3 eggs, 3.9% received 4 eggs and 3.7% received 5–19 eggs). However, the mapping of the comb content shows that only 1.8 ± 2.3 per cent (mean \pm s.d.,

Table 1. Repeated-measures ANOVA of log (number of eggs + 1) and *post hoc* Tukey HSD test for unequal sample size.

factor	statistic	p-value
treatment	$F_{2,87} = 12.074$	0.000024
	<i>post hoc</i> : brood removed versus control	0.00018
	<i>post hoc</i> : brood removed versus alpha removed	0.0073
	<i>post hoc</i> : control versus alpha removed	0.775
status	$F_{2,87} = 25.134$	0.000001
	<i>post hoc</i> : (new) alpha versus subordinates	0.00011
	<i>post hoc</i> : (new) alpha versus workers	0.00011
	<i>post hoc</i> : subordinates versus workers	0.318
time (repeated sessions)	$F_{5,435} = 4.027$	0.0014
	<i>post hoc</i> : session 1 versus sessions 2, 3, 5 and 6	<0.019
treatment \times status	$F_{4,87} = 1.366$	0.252
treatment \times time	$F_{10,435} = 3.734$	0.00008
status \times time	$F_{10,435} = 9.930$	0.000001

$n = 32$ colonies) of egg-containing cells had more than one egg (1.8% in control and 1.2% in both brood- and alpha-removed colonies; median values, Kruskal–Wallis ANOVA: $H_{2,32} = 0.810$, $p = 0.667$). The dramatic difference between very frequent multiple ovipositions and rare presence of multiple eggs in cells unambiguously demonstrates the intensity of egg replacement.

As was the case for the number of eggs laid, the index of egg replacement follows the same pattern in all treatments, albeit with a higher magnitude in brood-removed colonies (figure 3). Within each treatment, (new) alpha benefited from egg destruction at the expense of subordinates and workers. Subordinates and workers suffered equally from egg destruction, except in brood-removed colonies where workers suffered more. Between treatments, alpha benefited more from egg destruction in brood-removed than in control colonies. Consistently, workers suffered more in brood-removed than in control colonies (figure 3). These results show that alpha-removed colonies did not differ from control colonies, while they differed from brood-removed colonies. Following the removal of alpha, the new alpha destroyed and replaced other individuals' eggs as efficiently as alpha did.

Therefore, (new) alpha lays more eggs than subordinates and workers, and she further increases her share of reproduction by egg replacement. This is clear when comparing the share of all the eggs laid during the experiment ('gross' share) with the share of eggs surviving replacement ('net' share). Pooling the three treatments, the share of (new) alpha increased from a gross share of 58 ± 24 per cent to a net share of 71 ± 21 per cent (paired t -test: d.f. = 31, $t = -7.382$, $p < 0.0001$), while the share of subordinates decreased from 15 ± 14 per cent to 11 ± 13 per cent (d.f. = 31, $t = 4.532$, $p < 0.0001$) and that of workers decreased from 27 ± 24 per cent to 18 ± 19 per cent (d.f. = 31, $t = 5.584$, $p < 0.0001$). This is also mostly the case within treatments (Wilcoxon signed-rank tests, seven comparisons significant at $p < 0.05$ and two comparisons not significant at $p = 0.062$ and $p = 0.237$).

(e) Effect of colony size

The number of eggs laid was weakly correlated with colony size in some treatments. The correlation was significant for (new) alpha in brood-removed colonies (Pearson's $r = 0.67$, $p = 0.012$), close to significant in alpha-removed colonies ($r = 0.70$, $p = 0.054$), and not significant in control colonies

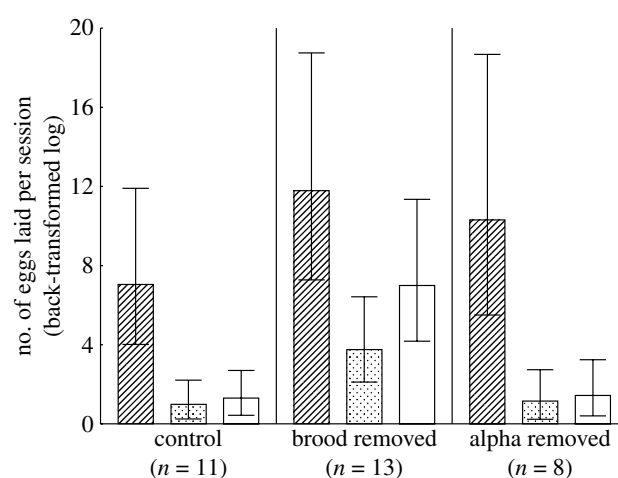


Figure 2. The number of eggs laid per session (mean \pm 95% CI). Hatched bars, (new) alpha; dotted bars, subordinates; open bars, workers. See table 1 for statistics.

($r = 0.43$, $p = 0.189$; figure 4a). However, these coefficients of correlation were not significantly different from each other (control versus brood removed: $p = 0.468$, control versus alpha removed: $p = 0.489$, alpha removed versus brood removed: $p = 0.928$) and thus the relationship between colony size and egg-laying remains unclear for (new) alpha. There was no significant correlation for subordinates in any of the three treatments (control: $r = -0.07$, $p = 0.842$; brood removed: $r = 0.43$, $p = 0.146$; alpha removed: $r = 0.26$, $p = 0.527$; figure 4b). However, while there was no significant correlation for individual workers in control and alpha-removed treatments ($r = 0.31$, $p = 0.352$ and $r = -0.35$, $p = 0.398$, respectively), there was a strong and highly significant correlation in brood-removed colonies ($r = 0.82$, $p = 0.0006$; figure 4c). This latter coefficient of correlation differed from the former two (control versus brood removed: $p = 0.060$, alpha removed versus brood removed: $p = 0.014$, control versus alpha removed: $p = 0.353$).

4. DISCUSSION

In *P. dominulus*, co-nesting foundresses may be related or not, which affects the potential reproductive conflicts. Our genetic analysis reveals a mean relatedness of 0.23 between co-nesting foundresses at the late founding stage, just prior to worker emergence, in a population

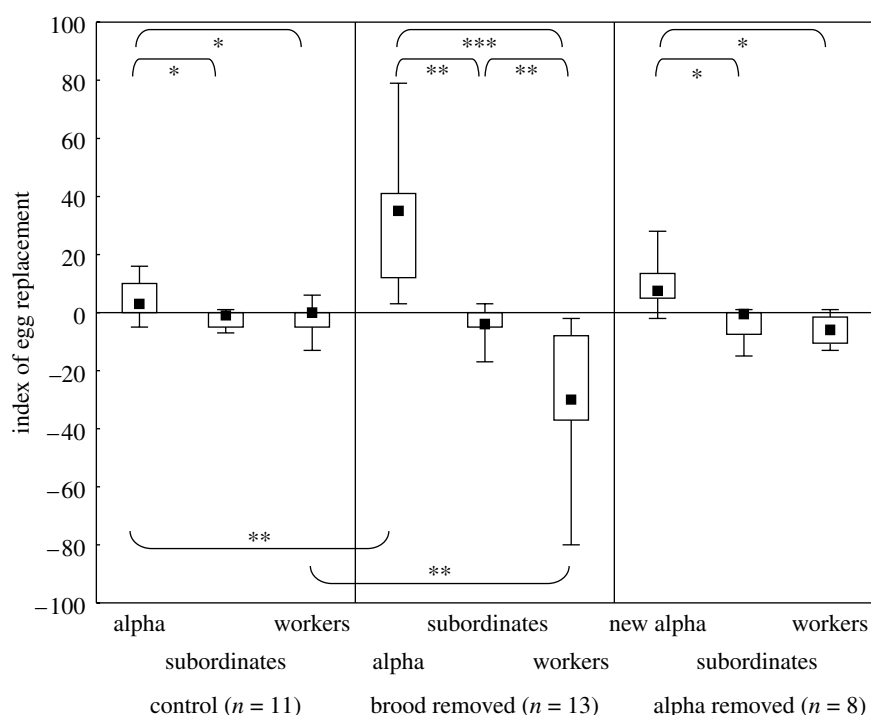


Figure 3. Index of egg replacement (median \pm quartiles and range) over the entire experiment (six sessions pooled). A positive index reflects an increase in the share of eggs, while a negative index indicates a decrease. Within each treatment, social status is compared within colonies by means of Friedman ANOVA and Wilcoxon signed-rank tests (top brackets). Between treatments, social status is compared by Kruskal–Wallis ANOVA and *post hoc* tests (bottom brackets). *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

near Florence, Italy. This is comparable with the results obtained in other populations of the same species at the same late founding stage. In another population near Florence, mean relatedness in two consecutive years was 0.31 and 0.21 (Queller *et al.* 2000, ‘late foundress’ stage), and in two sites of a population near Cadiz, Spain, it was 0.19 and approximately 0.29 (Zanette & Field 2008; ‘stable nests’ stage). The three studies each reveal a bimodal distribution of relatedness, with one peak at approximately 0 and another at approximately 0.75 (unrelated and full-sister foundresses, respectively). We found that 81 per cent of colonies contained unrelated foundresses (58% contained unrelated foundresses only and 23% contained a mixture of unrelated and full-sister foundresses), which is equivalent to approximately 80 per cent found in the Spanish population (Zanette & Field 2008). Queller *et al.* (2000) found that ‘only’ 35 per cent of foundresses were unrelated and 9 per cent were cousins, but this was when pooling colonies over seasons. Both Queller *et al.* (2000) and Zanette & Field (2008) showed that relatedness changes over time, and that it is the lowest at the late founding stage, when unrelated foundresses are more common. These results highlight that the potential conflict over colony inheritance is both very common and severe: in most *P. dominulus* colonies, alpha and workers are at risk of obtaining zero fitness if an unrelated foundress inherits the colony.

We found a strong correlation between colony size and worker egg-laying in brood-removed colonies. Workers produce very few eggs in small colonies, while they lay massively in larger colonies (up to 400 eggs over the duration of the experiment). Although this is merely a correlation, it suggests that worker reproduction may be prohibitively costly in small colonies. This may be because small colonies suffer proportionally higher productivity

costs for each worker reproducing and presumably working less (e.g. foraging less), because each worker represents a larger fraction of the total workforce in smaller colonies. This is supported by the fact that, at the founding stage, a smaller proportion of co-foundresses has developed ovaries in smaller associations (Cant & English 2006). Similarly, in several ant species, the number of individuals entering the dominance hierarchy and competing for reproductive rights decreases as colony size decreases (Monnin & Ratnieks 1999; Monnin *et al.* 2003; Molet *et al.* 2005; Heinze 2008).

Our analysis of the patterns of egg-laying and egg replacement shows that there is no actual conflict over colony inheritance between workers and subordinates, even though there is a potential conflict. Both the patterns of egg-laying and egg replacement show that a subordinate inherits the colony when alpha is removed, and that workers do not oppose this. The inheriting subordinate (new alpha) becomes as fertile as alpha, other subordinates and workers do not lay more eggs when the new alpha has inherited the colony, and the new alpha replaces subordinates’ and workers’ eggs as efficiently as alpha. Therefore, the new alpha fully inherits the colony upon the removal of alpha, without increased conflict with nest-mates. This was unexpected. There is no good evidence of nepotism in social insects (e.g. Strassmann *et al.* 2000; Tarpy *et al.* 2004; Châline *et al.* 2005; Holzer *et al.* 2006; but see Wenseleers 2007), and it is unclear whether *P. dominulus* workers can estimate relatedness to (new) alpha and subordinates (Dani *et al.* 2004; Dapporto *et al.* 2004). But workers do not necessarily need to estimate relatedness, because they should always prefer a worker inheriting the colony rather than a foundress (Queller *et al.* 1997). Owing to the high frequency of unrelated foundresses and the high threat to inclusive fitness they

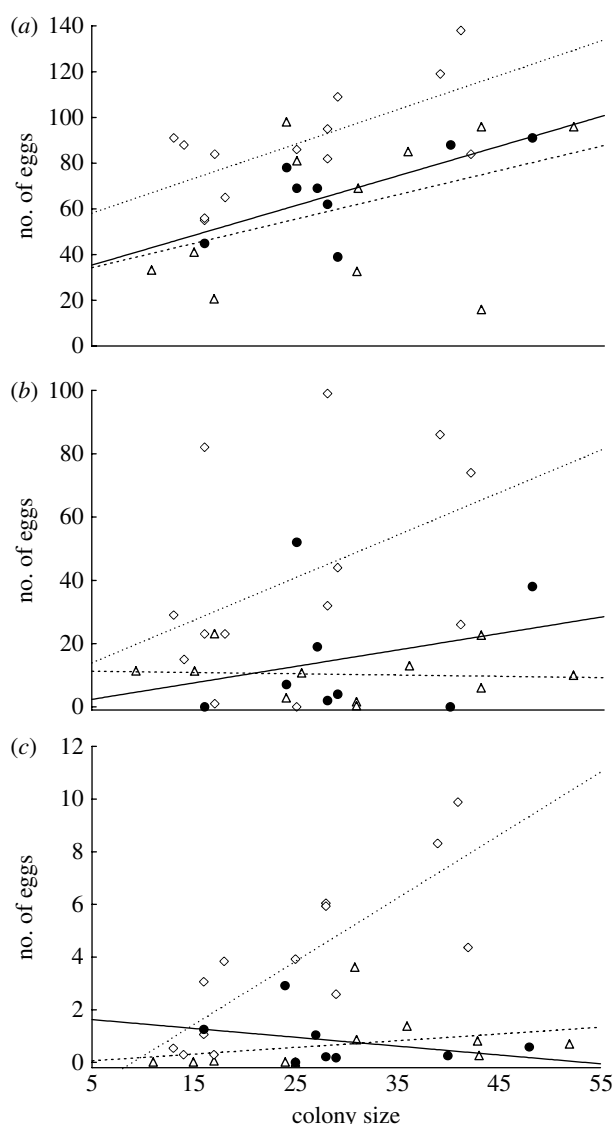


Figure 4. Cumulative number of eggs laid over the entire experiment as a function of colony size at the end of the experiment, for (a) (new) alpha, (b) subordinates and (c) individual workers (i.e. the number of eggs laid by workers divided by colony size). Triangles and dashed lines ($n=11$), control; diamonds and dotted lines ($n=13$), brood removed; circles and solid lines ($n=8$), alpha removed.

represent, one could expect workers to expel all subordinate foundresses. Indeed, foundresses are potentially identifiable by their behaviour (e.g. Reeve 1991) and cuticular chemical cues (Sledge *et al.* 2001). Thus, why do workers accept a subordinate as replacement breeder?

One possible explanation is that workers do not have the power to prevent a subordinate from inheriting the colony (Beekman *et al.* 2003). In natural conditions, subordinates disappear from the nest around the time of worker emergence, and it is likely that they are somehow forced to leave (Pfennig & Klahn 1985; Hughes & Strassmann 1988; Reeve 1991; Reeve *et al.* 1998; Gamboa *et al.* 1999). But it is unclear whether this is because of alpha or workers. It may be that alpha is physically capable of expelling subordinates, given that she dominates them, while workers are not. However, this would be surprising, given that subordinates are outnumbered by workers who are only slightly smaller in size (there is no morphological caste in *P. dominulus*, and although first-brood workers

tend to be small workers, subordinates also tend to be small foundresses; Röseler 1991).

Another possibility is that colony-level costs preclude workers from replacing subordinates at an early stage of colony growth, and that workers delay their response. Workers may momentarily accept a subordinate if worker reproduction would excessively hamper colony growth. In our experiment, the new alpha rapidly became as fertile as alpha, which contrasts with what occurs when alpha was removed in single-foundress colonies (Strassmann *et al.* 2004). In that case, one worker became dominant, but she needed approximately two to four weeks to fully develop her ovaries. As a consequence, colony growth was significantly reduced. This delay for workers to become fertile and the associated cost in reduced colony growth may result in workers momentarily accepting a subordinate. They may then expel subordinates later in the season and seek direct fitness benefits by producing males. Alternatively, workers may abscond the nest (Tibbetts 2007). Male production by workers could give rise to alternative reproductive strategies in the colonies that have been inherited by a (possibly unrelated) subordinate relative to colonies that have retained alpha or have been inherited by a (full-sister) worker, with workers producing more males in the former. Our experiment was carried out in the laboratory, and this may have altered how workers reacted to the removal of alpha. Workers could not mate, which could have prevented them from expelling and replacing subordinates, and workers could not leave the nest to start a colony of their own. However, workers becoming breeder needed at least two to four weeks to mate in the field (Strassmann *et al.* 2004), so that workers' inability to mate in our experimental set-up is not unrealistic.

Unrelated subordinate foundresses need to inherit the colony to obtain some fitness, and this can only be achieved at the expense of alpha and her daughters. That is, subordinates bet on alpha dying so that they can inherit the colony and reproduce with the help of alpha's daughters. At the founding stage, alpha benefits from the presence of subordinates because she monopolizes reproduction, while subordinates behave as workers. However, when workers emerge, subordinates are no longer essential, but they still represent a threat to alpha's fitness, so that alpha and/or her daughters would benefit from expelling them. This differs from stenogastrine wasps, as while colony inheritance is at the expenses of alpha and workers in *Polistes* (they get zero fitness when an unrelated subordinate becomes the new alpha), it is not so in stenogastrines. On the contrary, in the latter species, colony inheritance provides posthumous benefits to the deceased alpha. Stenogastrines queue for reproduction, and when alpha disappears, the wasp next in the queue becomes dominant and rears both the offspring of the previous alpha and her own. Because stenogastrine colonies are not annual, these posthumously reared offspring may become reproductive and yield some fitness (Landi *et al.* 2003; Field *et al.* 2006; Bridge & Field 2007). Also, stenogastrine nest-mates are related, so that the deceased alpha will get additional fitness through the new alpha (Landi *et al.* 2003; Field *et al.* 2006; Bridge & Field 2007). Our experiment suggests that, in *P. dominulus*, the time window for subordinates to inherit the colony is relatively long, with subordinates inheriting the colony up to several weeks following worker emergence.

This relatively high prospect of colony inheritance helps explain why subordinate foundresses commonly associate with an unrelated dominant foundress yielding them little direct fitness: they bet on her death and await their hour.

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